

**Spectroscopic Characterization and Antibacterial Activity of Synthesized Schiff Bases**

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ABSTRACT

In this Study, three different Schiff bases were synthesized from aldehyde (benzaldehyde) and primary amines (aniline, 2-amino benzoic acid and o-phenylenediamine) using Hugo Schiff method reported in the literature [1]. The Schiff bases were then purified with ethanol and characterized by FTIR spectroscopy. The results of the FTIR characterization shows IR absorption bands within a particular regions which indicate the presence of the functional group "imine" and does not show absorbance in the carbonyl (C=O) region. Also, the synthesized Schiff bases were subjected to antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* and the test was carried using agar well diffusion method. The results shows that the Schiff bases 'D' (*O-phenylenediamine Benzaldimine*) was not active against *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* but was active against *Escherichia coli* and *Staphylococcus aereus*. Similarly, 'C' (*Benzaldimine*) was the most active against *Klebsiellapneumoniae* but not active against *Escherichia coli*. Also the Schiff base 'E' (*Anthranilic acid Benzaldimine*) was active against *Escherichia coli*, *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* but shows low level of activeness against *Staphylococcus aereus*. The activity of the synthesized Schiff bases depends on the type of the bacteria and other functional groups present at the ortho position of the "imine" group.

Keywords: Schiff bases, Spectroscopy, Antibacterial, Synthesis and Characterization.

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INTRODUCTION:**Schiff Bases**

Schiff bases are compounds with general formula $RHC=NR'$ ($R' = \text{Alkyl or Aryl}$). They can be considered a sub- class of imines, being either secondary ketimines or secondary aldimines depending on their structure. They are condensation products of ketones or aldehydes (aldehydes and ketones) with primary amines and were first reported by Hugo Schiff in 1864 [2]. Formation of Schiff bases generally takes place under acids or bases catalysis. The common Schiff bases are crystalline solids, which are feebly basic but at least some form insoluble salts with strong acids [1]. Schiff bases are used as intermediates for the synthesis of amino acids or as ligands for preparation of metal complexes having a series of different structures. Schiff bases are the compounds containing azomethine group ($-HC=N-$)[3].

A Schiff base behaves as a Flexi-dentate ligand and commonly co-ordinates through the O atom of the de - protonated phenolic group and the N atom of azomethine group [4]. In Schiff base azomethane nitrogen and other donor atoms like oxygen play a vital role in co-ordination chemistry, hence an attempt is made to study the interaction of reduced Schiff base with transition of metals biological interest and to investigate the co-ordination chemistry of such interactions [5].

Aromatic aldehydes especially with an effective conjugation system, form stable Schiff bases, where as those of aliphatic aldehydes are unstable and readily polymerize [6]. Schiff base ligands with aldehydes are formed more readily than with ketone (carbonyl carbon). Schiff bases have very flexible and different structures. Schiff bases are generally *bi*, *tri*, or *tetra*-dentate chelate ligands and form very stable complexes with metal ions. Their chemical and physical properties in various field such as preparative uses, identification, or protection and determination of aldehyde or ketones, purification of carbonyl and amino compounds or production of these compounds in complex or sensitive reactions have been studied by various workers [7]. Schiff base ligands have significant importance in chemistry, especially in the development of Schiff base complexes, because Schiff base complexes are potentially capable of forming stable complexes with metal ions. Many Schiff base complexes show excellent catalytic activity in various reactions at high temperature and in the presence of moisture. Over the past few years, there have been many reports on their applications in homogeneous and heterogeneous catalysis [8].

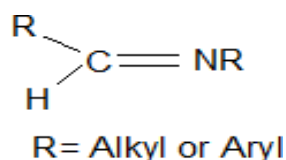
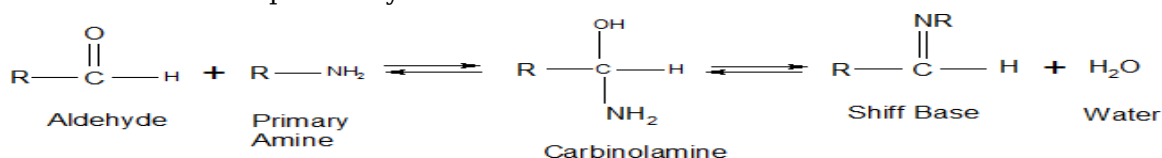


Fig. 1: General structure of a Schiff base

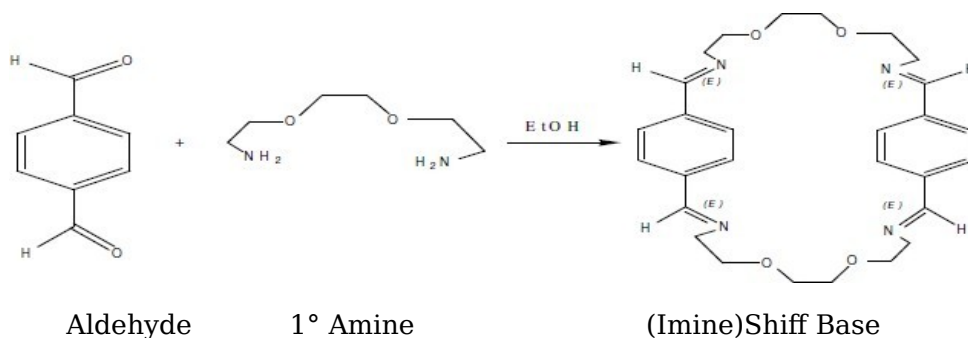
Formation of Schiff Base

The formation of a Schiff base from an aldehyde (or ketone) is a reversible reaction and generally takes place under acid (or base) catalysis, or upon heating. The reaction is generally driven to completion by separation of the product or removal of water, or both. Many Schiff bases can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base [9].

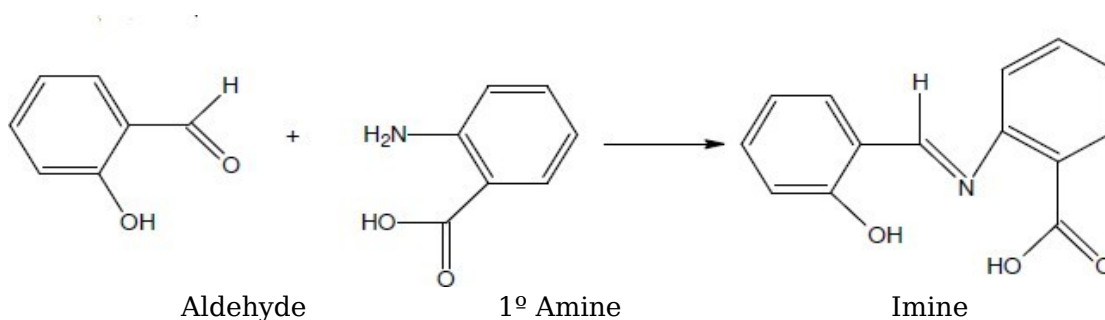
Below are some examples of Synthesized Schiff bases:-



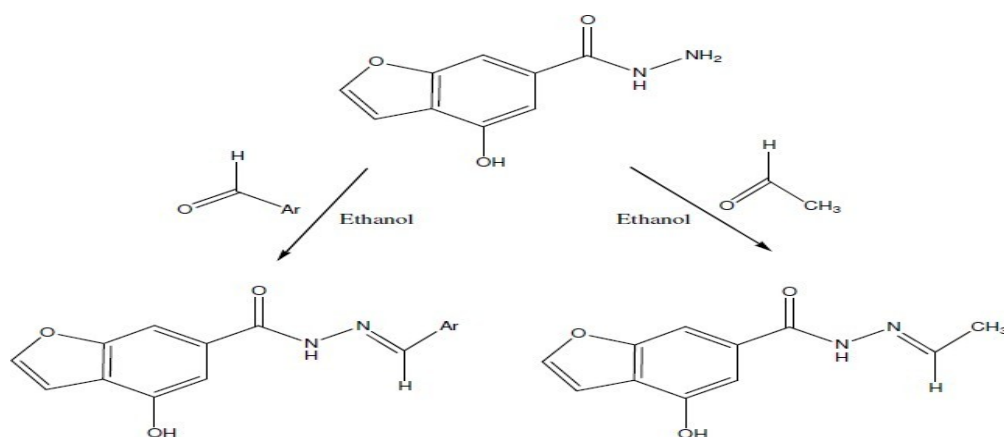
Scheme 1: Schiff base by [8]



Scheme 2: Prepared Schiff base formation by [10]



Scheme 3: Schiff base Synthesized by [10]

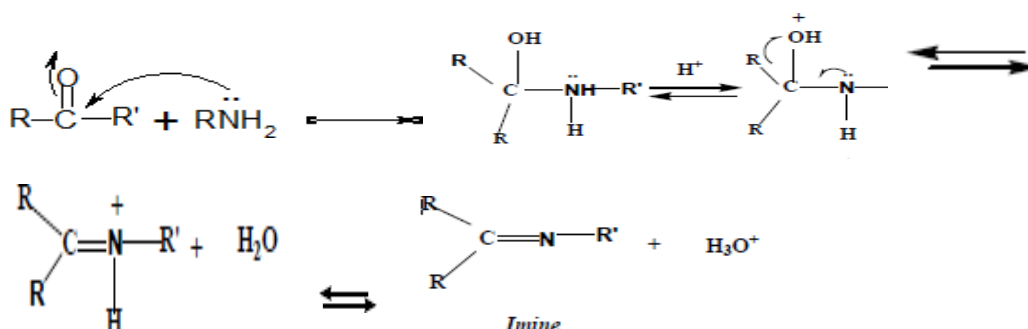


R= Aryl

R=Alky

Scheme 4: Synthesized Schiff bases by [10]

Mechanism of Schiff Base Formation



Scheme 5: Mechanism of Schiff base formation

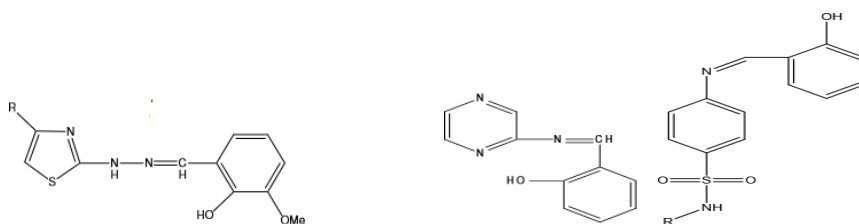
The mechanism of Schiff base formation is another variation on the theme of nucleophile addition to the carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration. Iminium salt ($R_2C=N^+R_2$) at the other extremes are very rapidly hydrolyzed by water and have to be prepared under rigorously anhydrous conditions.

The dehydration of carbinolamine is also catalyzed by base. This reaction is somewhat analogous to the **E2** elimination of alkyl halides except that it is not a concerted reaction. It proceeds in two steps through an anionic intermediate. The Schiff base formation is really a sequence of two types of reactions, i.e. **addition** followed by **elimination**, yet the acid concentration cannot be too high because amines are basic compounds. If the amine is protonated and becomes non-nucleophile, equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore, many Schiff bases synthesis are best carried out at mild acidic pH.

Biological Importance of Schiff bases

Schiff bases have a large number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylation agent to the carbon-nitrogen double bond. Reactions of this type have been put to good use in natural product synthesis [11]. Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving

interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine such as 2-hydroxy-3-methoxybenzylidenethiothiazole[12]. Stereochemical investigation carried out with the aid of molecular model showed that Schiff base formed between methylglyoxal and the amino group of the lysine side chains of proteins can bent back in such a way towards the N atom of peptide groups that a charge transfer can occur between these groups and oxygen atoms of the Schiff bases [13]. In this respect pyridoxal Schiff bases derived from pyridoxal and amino acids have been prepared and studied from the biological point of view. Osman [14], prepared thiadiazole derivative compounds of salicylaldehyde which were found to be highly



potent antibacterial against *Bacillus cereus* and antifungal against *Aspergillus niger*. Several compound incorporating piperazinyl guanidine when condensed with Salicylaldehyde were found to exhibit cardiovascular and vasodepressive antimicrobial activity, a typical example is methoxybenzylidenethiothiazole.

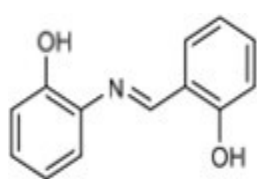
(a) methoxybenzylidenethiothiazole salicyldimine

(b) 2-aminopyrazine (c) p-sulfonamidesalicyldimine

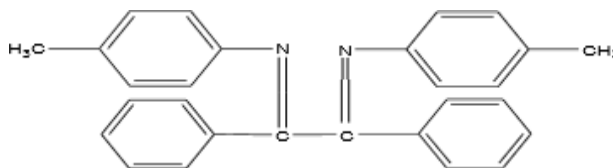
Scheme 6: Some synthesized Schiff Bases

Biological activity

Schiff bases are characterized by an imine group $-N=CH-$, which helps to clarify the mechanism of transamination and racemization reaction in biological systems. It exhibits antibacterial and antifungal effect in their biological properties examples are: 2-hydroxysalicyldimine and P-Toludinebenzilimine [15]. Metal-imine complexes have been widely investigated for antitumor and herbicidal use. They can work as models for biologically important species [16].



(a)



2-hydroxysalicyldimine

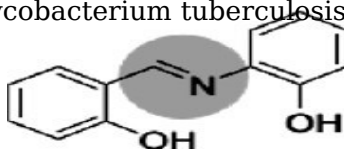
(b) P-

Toludinebenzilimine Scheme 7: Schiff

Bases

Antibacterial properties

Mortality increase caused by infectious diseases is directly related to the bacteria that have multiple resistance to antibiotics. The development of new antibacterial drugs enriched by innovatory and more effective mechanisms of action is clearly an urgent medical need [17]. Schiff bases are identified as promising antibacterial agents. For example, N- (Salicylidene)-2-hydroxyaniline is active against *Mycobacterium tuberculosis* [18].

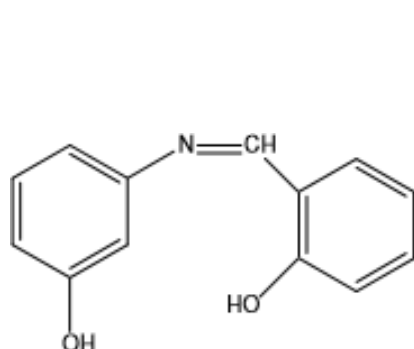


Scheme 8: N-(Salicylidene)-2-hydroxyaniline as the example of

bioactive Schiff base Antifungal properties

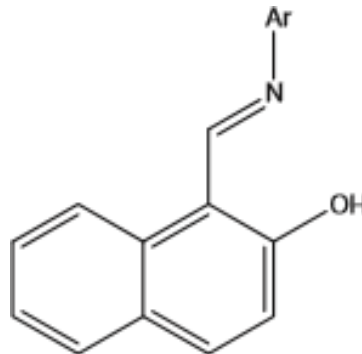
Fungal infections usually are not only limited to the contamination of surface tissues. Exploration and development of more effective antifungal agents is a necessity, and the individual Schiff bases are considered to be promising antifungal medicines [8] Such Schiff bases include; 3-hydroxysalicyldimine and 2-hydroxy-1-naphthaldimine. Some of them, such as imine derivatives of quinazolinones possess antifungal properties against *Candida albicans*, *Trichophytonrubrum*, *T. mentagrophytes*, *Aspergillusniger* and *Microsporumgypseum*. Schiff bases and their metal

complexes formed between furan or furylglyoxal with various amines exhibit antifungal activity against *Helminthosporiumgramineum*- causing leaf stripe in barley, *Syncephalostrumracemosus*- contributing to fruit rot in tomato and *Colletotrichumcapsici*-



causing anthracnose in chillies [20]

3-hydroxysalicyldimine



2-hydroxy-1-naphthaldimine

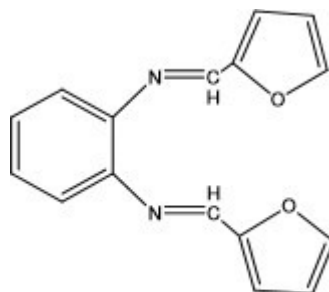
Scheme 9: Schiff Bases

Biocidal properties

Schiff bases obtained by the synthesis of o-aminobenzoic acid and β -keto esters have found biocidal use against *S. epidermidis*, *E. coli*, *B.cinerea* and *A. niger*[21]. By contrast, Schiff bases of is at in derivatives are used in the destruction of protozoa and parasites [22].

Application in Synthesis and Chemical Analysis

Schiff bases are a group of organic intermediates, which are very often used in the synthesis and chemical analysis. They are exerted in the production of pharmaceutical and agrochemical industry. In the reaction with hydrogen cyanide Schiff bases may form α -amino acid precursors (Strecker synthesis) [11]. Moreover, chiral Schiff bases are used as initial substrates for the asymmetric synthesis of α -amino acids, and as catalysts in asymmetric synthesis as in N,N'-o- phenylenediamine di[2-furacarbozaladimine] [16].



Scheme10: N, N'-o-phenylenediaminedi[2-furacarbozaladimine]

Furthermore, the imines obtained by the condensation reaction of arylamines and carbonyl compounds have determined a group of intermediates used in the preparation of important compounds (arenediazonium nitrates, N- arylarenecarboxamides, the appropriate amines and cyanamides, β -lactams) [15]. They are also used for the preparation of acyclic and macrocyclic compounds, such as: cryptats, coronates and podates [23]. These compounds lead to the formation of Ruhemann's purple (reaction between an amino acid and ninhydrin), which allows to detect and assist in the identification of fingerprints [24].

IR Characterization

When an infrared light is passed through a sample of organic compound, some of the frequencies absorbed and the absorbed or transmitted is plotted against frequency, the result is infrared spectrum, the excitation of molecular vibration and rotation gives rise to absorption in the infrared region of the spectrum. It was discovered that when an IR light is passed through an organic compound which has an imine functional group (schiffbase) it produce a sharp band at a region of $1643\text{-}1530\text{ cm}^{-1}$ which confirms the formation of Imines bond (-C=N-H) and the absences of carbonyl bond (C=O) due to the stretching vibration of the imines group at (-C=N-H). Ligands are also stable and have sharp melting point that indicate the

purity of the ligands. [11].

RESEARCH METHODOLOGY

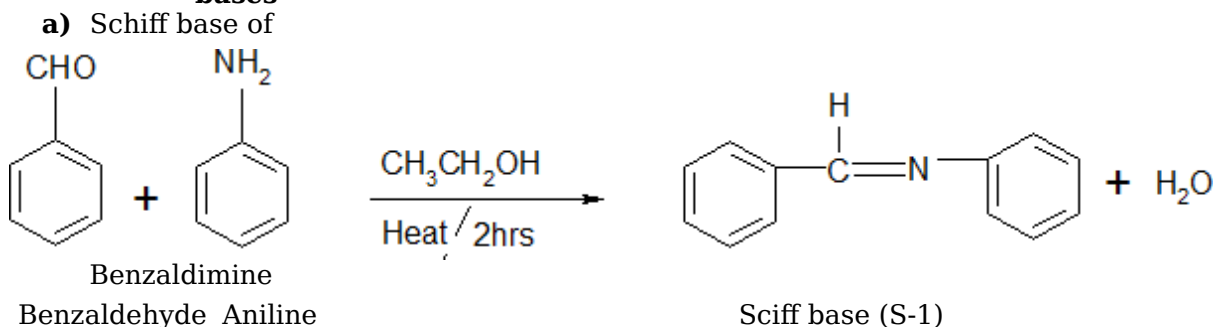
Materials

The materials used are Infrared spectroscopic machine and other necessary laboratory equipment such as; measuring cylinder, beaker, spatula, dessicator, funnel, conical flask, filter paper, magnetic stirrer bead, refluxing condenser, magnetic hot plate e.t.c.

Reagents

All reagents and solvents are of analytical grade and used without further purification. These include; benzaldehyde, aniline, o-phenylenediamine, 2-amino benzoic acid, ethanol. The reagents were obtained from the chemical store of the Department of Chemistry, ATBU Bauchi

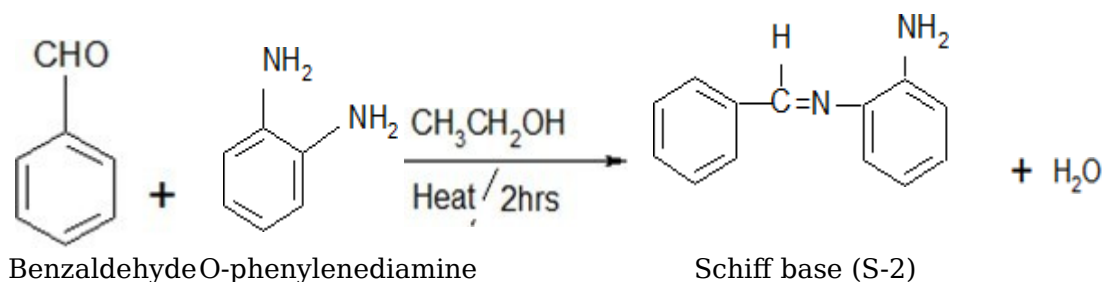
Preparation of Schiff bases



Scheme 11: preparation of Benzaldimine

Aniline (9.17 ml; 0.1 mol) was mixed with benzaldehyde (10.10 ml; 0.1 mol) in 30 ml of ethanol, and was heated under reflux for 2 hrs. The resultant solution was left over night to form sold particles. The solid product formed was filtered using simple filtration, purified by recrystallization from ethanol, washed with ethanol, and then dried in a dessicator to afford 11.8 g, 65% yield as orange crystals.

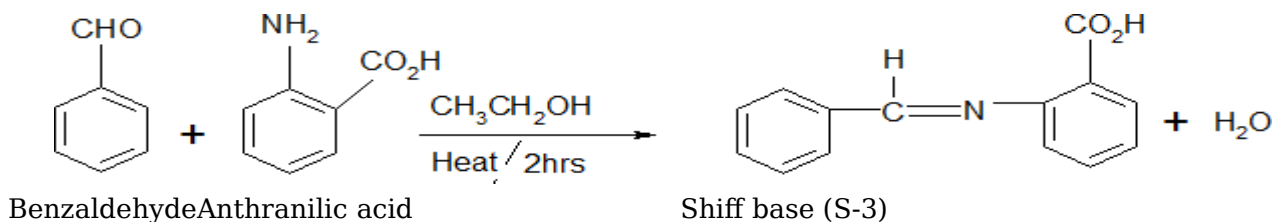
b) Schiff base of O-phenylenediaminebenzaldehyde



Scheme 12: Preparation of O-phenylenediaminebenzaldehyde

O-phenylenediamine (10.80 g; 0.1 mol) was mixed with benzaldehyde (10.10 ml; 0.1 mol) in 30 ml of ethanol, and was heated under reflux for 2 hrs.. Precipitate was formed immediately, and the solid product was filtered using simple filtration, purified by recrystallization from ethanol, washed with ethanol, and then dried in a dessicator to afford 15.7 g, 80% yield as light yellow crystals.

c) Schiff base of Anthranilic acid benzaldimine



Scheme 13: Preparation of Anthranilic acid benzaldimine

Anthranilic acid (13.70 g; 0.1 mol) was mixed with benzaldehyde (10.10 ml; 0.1 mol) in 30 ml of ethanol, and was heated under reflux for 2 hrs. Some solid particles was formed immediately, and the solid product was filtered using

simple filtration, purified by recrystallization from ethanol, washed with ethanol, and then dried in a dessicator to afford 14.0 g, 73% yield as milky color crystals.

Testing for purity

The purity of all the synthesized Schiff bases were tested by taking their respective melting point using melting point block and thermometer which they all gave a sharp melting point.

Characterization of the Schiff bases

The purified Schiff bases were characterized using the Fourier Transform Infrared Spectrophotometer (FTIR) at Bayero University Kano, Department of Chemistry. The assigned structures agree with the spectral data.

Antimicrobial Assay

This is the study of antimicrobial activity (sensitivity) of the synthesized Schiff bases against microorganisms. It helps to determine the therapeutic action of the Schiff bases.

Test Organisms

The test organisms used include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The choice of these organisms is based on their implication in human diseases such as diarrhea. The test organisms were obtained and sub-cultured at Almanzor Clinical Laboratory Bauchi and antimicrobial assay was carried out in ATBU microbiology laboratory.

Preparation of Nutrient Agar Medium

Nutrient agar medium was prepared according to the manufacturer's instruction. A weight of 5.6 g was dissolved in 200 ml of distilled water in a conical flask. It was boiled on a hot plate to homogenize and was then poured into petri-dishes and allowed to solidify.

Screening for antibacterial activity (Agar well diffusion method)

The antibiotic sensitivities of the Schiff base were tested against the selected bacterial cultures using agar diffusion method employed by Bauer, *et al.*, [25]. Stock cultures of the isolates were sub-cultured into sterile peptone water and were incubated for 18-24 hrs. From that, the standardized organisms (0.1 ml) each were poured into the petri-dishes, shaken to allow even distribution of the organisms. Wells (8 mm diameter) were bored into the agar after it cools using a sterile cork borer and 0.3 ml of the Schiff bases, antibiotics and the solvent as control were introduced into each well. The culture plates were incubated at 37°C for 24-72 hours, and the wells were assessed after each 24 hours.

The assessment of the antimicrobial activity was based on measurement of the diameter of the inhibition zones formed around the wells. Streptomycin was used as positive control and dimethylsulfoxide (DMSO) solvent was also used as negative control.

RESULTS AND DISCUSSION

Results

Synthesis of Schiff bases- The Schiff bases **C**, **D** and **E** were obtained from the reaction of benzaldehyde with aniline, O-phenylenediamine and 2-amino benzoic acid respectively in a 1:1 stoichiometric ratio. The nature, yield, molecular formula, melting point and colour of the synthesized Schiff bases are shown in table 1 below:

Table 1: Physical Properties and yields of the Synthesized Schiff Bases

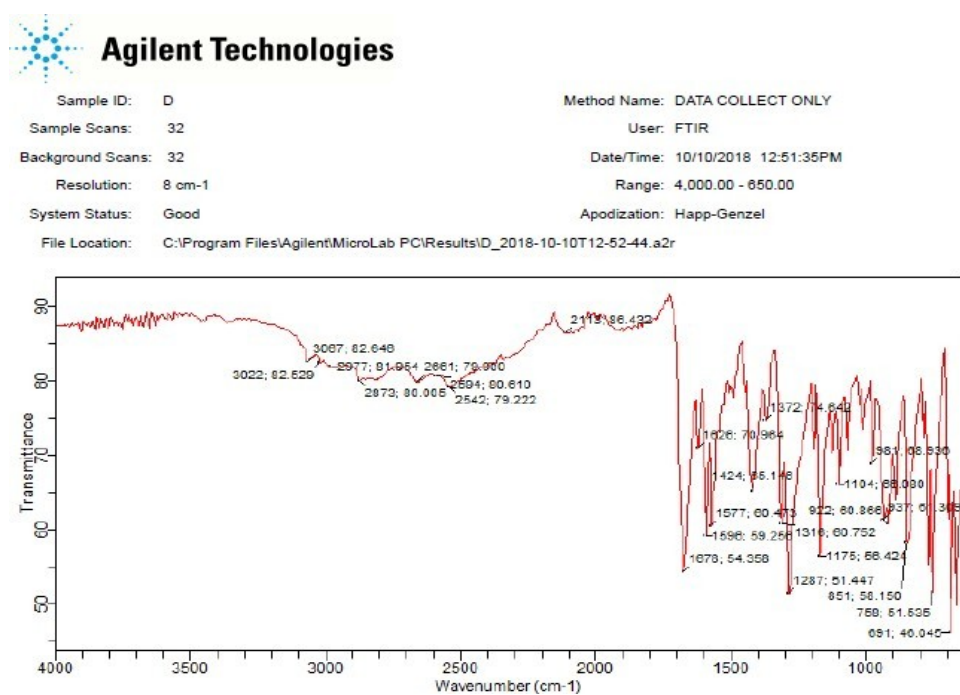
S/ NO	Schiff Base	Nature	Molecular Formula	Melting Point	Colour	% Yield

1.	Benzaldimine (C)	Fine crystals	$C_{13}H_{11}N$	88-91°C	Orang e	65
2.	O- phenylenediam ine Benzaldimine (D)	Soli d	$C_{13}H_{12}N_2$	110- 113°C	Light Yello w	80

3.	Anthranilic acid Benzaldimine (E)	Coarse crystals	C ₁₄ H ₁₁ N	90-98°C	Milky	73
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Table 2: FTIR values of the synthesized Schiff base

S/ NO	Schiff Base	IR-band (C=N)cm ⁻¹	IR-band (Ar-C-H)cm ⁻¹	IR-band (Ar-C=C)cm ⁻¹
1.	Benzaldimine (C)	1596	3067	2113
2.	O-phenylenediamine Benzaldimine (D)	1592	3182	2084
3.	Anthranilic acid Benzaldimine (E)	1599	3195	2121



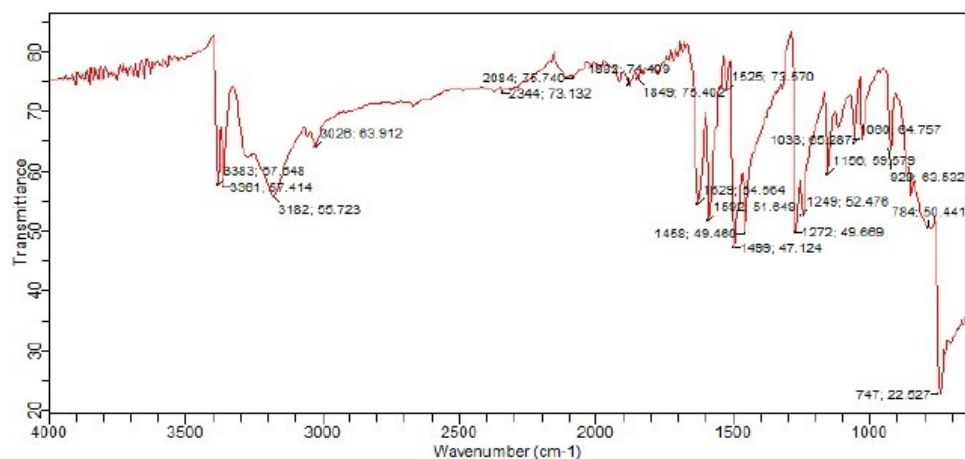
IR Absorption band for Benzaldimine



Agilent Technologies

Sample ID: B
Sample Scans: 32
Background Scans: 32
Resolution: 8 cm⁻¹
System Status: Good
File Location: C:\Program Files\Agilent\MicroLab PC\Results\B_2018-10-10T12-50-07.a2r

Method Name: DATA COLLECT ONLY
User: FTIR
Date/Time: 10/10/2018 12:48:35PM
Range: 4,000.00 - 650.00
Apodization: Happ-Genzel



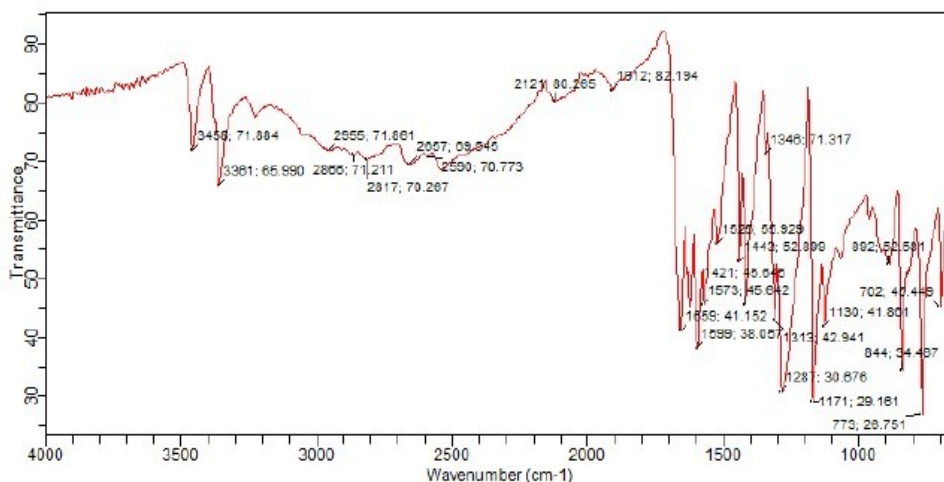
IR Absorption band for O-phenylenediaminebenzaldimine



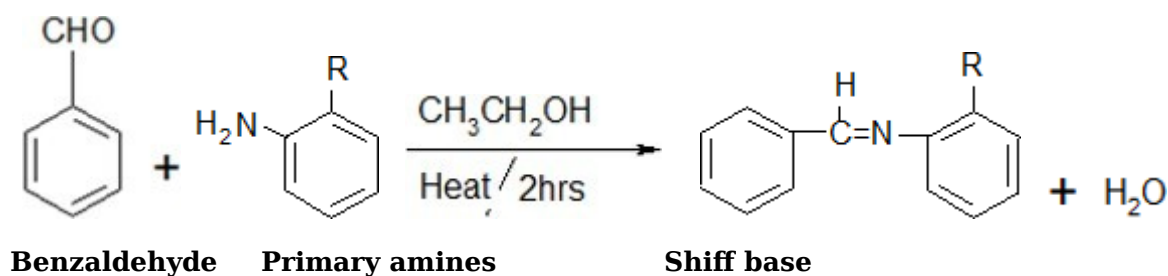
Agilent Technologies

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Range: 4,000.00 - 650.00
Apodization: Happ-Genzel



IR Absorption band for Anthranilic acid benzaldimine



Scheme 14: Synthesis of Schiff bases Table 3: Synthesis of Schiff bases

S/ NO	Schiff base	R
1.	C	-H
2.	D	-NH ₂
3.	E	-COOH

Antimicrobial assay

The antimicrobial assay of the synthesized Schiff bases were determined using the agar well diffusion method and the results are presented in table 4 below:

Table 4. Zone diameter of inhibition (mm) of bacterial growth produced by the Synthesized Schiff bases

Organism	A	B	C	D	E
<i>Escherichia Coli</i>	8	16	8	15	18
<i>Staphylococcus aureus</i>	18	16	14	8	
<i>Pseudomonas aeruginosa</i>	8	8	15	8	12
<i>KlebsiellaPneumoniae</i>	8	11	18	8	14

KEY:

A= DMSO

B=

Streptomycin

C=

Benzaldimine

D= O-

phenylenediaminebenzaldimine

E= Anthranilic acid

benzaldimine

Discussion of Results

Synthesis of Schiff bases

All the Synthesized Schiff bases follow the same mechanism for their formation that is, first is the nucleophilic attack from the amine as an acid base reaction forming "carbinolamine" intermediate, followed by proton transfer from the nitrogen, and then protonation with alcohol as solvent, followed by removal of water and finally deprotonation from the solvent forming imine functional group. When benzaldehyde reacts with the three substrates it produces three different Schiff bases "C, D and E" respectively as shown in table 3 which they differ in the

substituent of their ortho position leading to the different colour, nature, molecular formula and their activity. The Synthesized Schiff bases are stable and have sharp melting point ranges that indicate the purity of the Schiff bases. The IR spectrum of each Schiff base confirms the formation of Imines bond ($-\text{CH}=\text{N}-$) and the absence of carbonyl bond ($-\text{C}=\text{O}$) except for anthranilic acid benzilimine which shows as a result of carboxylic acid group that is attached to the benzene ring. (Table 2)

A strong to medium intensities bands are assigned to the carbon nitrogen double bond ($\text{C}=\text{N}$) with the stretching frequency range between $1587\text{--}1620\text{ cm}^{-1}$. In the aromatic C-H band has the region $3065\text{--}3195\text{ cm}^{-1}$, O-H band for benzoic acid at 3458 cm^{-1} , N-H band at 3381 cm^{-1} & 3361 cm^{-1} for amino group that is present at O-

phenylenediamminebenzaldimine alongside with the aromatic C=C bonds for each Schiff base. But for 2-amino benzoic acid it also shows strong sharp band at 1678 cm⁻¹ due to the presence of carbon double bond to oxygen (C=O) bond.

Antimicrobial Assay

The results for antibacterial activities of the synthesized Schiff bases (Table4) shows that Some Specific Synthesized Schiff bases was active against some of the tested microorganisms while not so active against others. It was found that 'C' is most active against *Klebsiellapneumoniae*, and relatively less active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but not active against *Escherichiacoli*. Similarly, 'E' is most active against *Escherichiacoli* and relatively less active against *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* but not active against *Staphylococcus aureus*. However, the Schiff base 'D' is found to be not active against *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* but active against *Escherichiacoli* and *Staphylococcus aureus*.

CONCLUSION

The result of this investigation revealed that the synthesized Schiff bases have antimicrobial activities against some tested bacteria which can be used to cure some bacterial diseases.

RECOMMENDATION

The antimicrobial activities of the synthesized Schiff bases may serves as a get way to a new drugs that will be synthesized to cure microbial diseases which may have less site effect than the existing drugs.

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